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ORIGINAL PAPER

Genetic structure of *Aegilops cylindrica* Host in its native range and in the United States of America

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Abstract Chloroplast and nuclear microsatellite markers were used to study genetic diversity and genetic structure of *Aegilops cylindrica* Host collected in its native range and in adventive sites in the USA. Our analysis suggests that *Ae. cylindrica*, an allotetraploid, arose from multiple hybridizations between *Ae. markgrafii* (Greuter) Hammer. and *Ae. tauschii* Coss. presumably along the Fertile Crescent, where the geographic distributions of its diploid progenitors overlap. However, the center of genetic diversity of this species now encompasses a larger area including northern Iraq, eastern Turkey, and Transcaucasia. Although the majority of accessions of *Ae. cylindrica* (87%) had D-type plastomes derived from *Ae. tauschii*, accessions with C-type plastomes (13%), derived from *Ae. markgrafii*, were also observed. This corroborates a previous study suggesting the dimaternal origin of *Ae. cylindrica*. Model-based and genetic distance-based clustering using both chloroplast and nuclear markers indicated that *Ae. tauschii* ssp. *tauschii* contributed one of its D-type plastomes and its D genome to *Ae. cylindrica*. Analysis of genetic structure using nuclear markers suggested that *Ae. cylindrica* accessions could be grouped into three subpopulations (arbitrarily named N-K1, N-K2, and N-K3). Members of the N-K1 subpopulation were the most numerous in its native range and members of the N-K2 subpopulation were the most common in the USA. Our analysis also indicated that *Ae. cylindrica* accessions in the USA were derived from a few founder genotypes. The frequency of *Ae. cylindrica* accessions with the C-type plastome in the USA (~24%) was substantially higher than in its native range of distribution (~3%) and all C-type *Ae. cylindrica* in the USA except one belonged to subpopulation N-K2. The high frequency of the C-type plastome in the USA may reflect a favorable nucleo-cytoplasmic combination.

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Introduction

Jointed goatgrass (*Aegilops cylindrica* Host; $2n = 4x = 28$; genome CCDD), an allotetraploid of the Triticeae tribe (Poaceae family), formed through amphidiploidization of a hybrid or hybrids between *Ae. tauschii* Coss. ($2n = 2x = 14$; genome DD) and *Ae. markgrafii* (Greuter) Hammer (syn. *Ae. caudata* L.; $2n = 2x = 14$; genome CC) (Chennaveeraiah 1960; Jaaska 1981; Johnson 1967; Kihara and Matsumura 1941). Although studies on phenotypic (Maan 1976; Tsunewaki 1996) and organellar DNA variation among alloplasmic lines of wheat (Ogihara and Tsunewaki 1988; Wang et al. 2000; Wang et al. 1997) suggested cytoplasmic homology between *Ae. cylindrica* and *Ae. tauschii* (D-type cytoplasm), a more recent analysis with chloroplast microsatellite markers has shown that both *Ae. tauschii* (D-type cytoplasm) and *Ae. markgrafii* (C-type cytoplasm) have contributed their cytoplasms to *Ae. cylindrica* (Gandhi et al. 2005).

The geographic distribution of *Ae. cylindrica* encompasses and extends beyond areas, where its diploid progenitors, *Ae. tauschii* and *Ae. markgrafii*, can be found (Fig. 1). *Ae. cylindrica* has spread westward to Greece, Bulgaria, Romania, Kosovo, Montenegro, Serbia, and Hungary. To the east, *Ae. cylindrica* is found in central Asia. Northwards, it is present in the Caucasus region and along the Black Sea coast. Though rare, this species is also present in the western arc of the Fertile Crescent involving Lebanon, Jordan, Syria, northern Iraq, and northwestern Iran. *Ae. cylindrica* is also adventive in many parts of Europe, Asia, and America (Slageren 1994).

Throughout its range of distribution, *Ae. cylindrica* is considered a weedy species, particularly in common wheat (*Triticum aestivum* L.), where it chronically infests fields in the Mediterranean, the Middle East, Europe, and the United States of America (USA) (Dewey 1996; Ogg and Seefeldt 1999; Slageren 1994). Jointed goatgrass has also been suggested as a source of genetic variation for wheat improvement (El Bouhssini et al. 1998; Farooq et al. 1992; Iriki et al. 2001) because it is a close relative of common wheat—both species carry the D genome donated by *Ae. tauschii* (Kimber and Zhao 1983; Riley and Law 1965). In addition, natural hybridization between wheat and jointed goatgrass suggests a potential for gene flow between these species under field conditions (Gandhi et al. 2006; Zemetra et al. 1998). Thus, there is considerable interest in understanding various aspects of the evolution of *Ae. cylindrica* for its better management and use.

Here, we present an analysis of the genetic structure of jointed goatgrass from its native range and parts of the USA. This analysis provides insights on the formation of

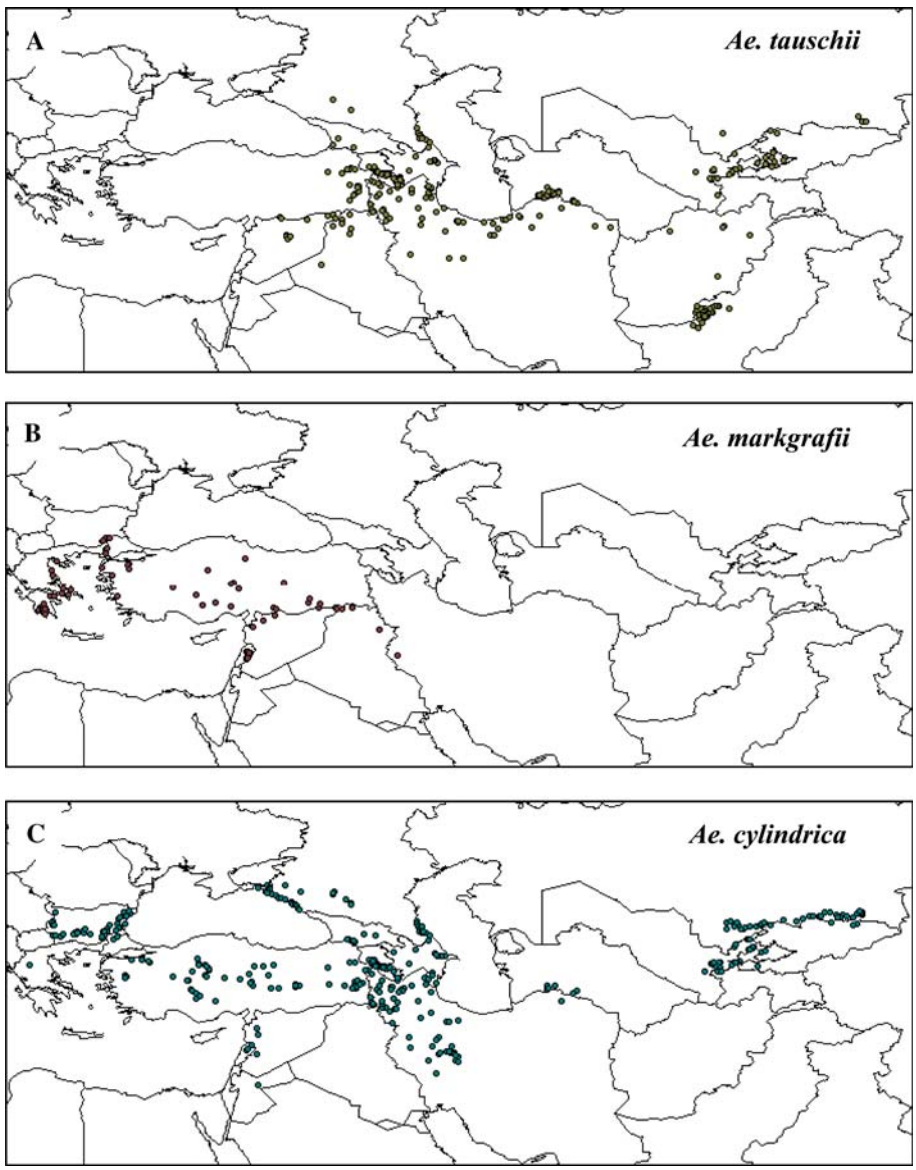

Fig. 1 Maps showing the distribution of collections of *Ae. tauschii* (**a**), *Ae. markgrafii* (**b**), and *Ae. cylindrica* (**c**). The maps display collection sites for material held by the International Center for Agricultural Research in the Dry Areas (ICARDA; http://www.icarda.org/). The geographic coordinates were obtained form the system-wide information network for genetic resources (SINGER; http://singer.cgiar.org/)

Ae. cylindrica and the relationship between adventitious accession *Ae. cylindrica* from the USA and those from its native range.

Materials and methods

Plant material

One hundred and seventy-three *Ae. cylindrica* accessions were analyzed using nuclear and chloroplast microsatellite markers (a list of accessions and collection sites is presented in the supplementary Table 1). These accessions were collected from 18 countries covering parts of the native and non-native distribution of *Ae. cylindrica*. Five *Ae. tauschii*, three *Ae. markgrafii*, and one *T. aestivum* accessions were also included as outgroups in the assays. For chloroplast microsatellite marker analyses, an additional 15 *Ae. tauschii* and 6 *Ae. markgrafii* accessions were used. The *Ae. cylindrica* collection sites from its native and non-native distribution in the USA were arbitrarily divided into 10 geographic regions (Fig. 2). The R1 region encompassed sites from Eastern Europe (western edge of Turkey, Bulgaria, Greece, and Serbia and Montenegro), whereas the R2 region included sites from central Turkey. The R3 region included sites from the Levant (Syria, Lebanon, and Jordan). The R4 region included collection sites from Georgia, Armenia, Azerbaijan, and Daghestan, whereas the R5 region had sites from eastern Turkey and northern Iraq. The R6 region had accessions from northwestern Iran. The R7 region included sites from Central Asia (Northeastern Iran, Turkmenistan, Uzbekistan, Afghanistan, Tajikistan, and Kyrgyzstan) and China. The US1 region included sites from the Great Plains of the USA (Nebraska, Oklahoma, Colorado, Kansas, Wyoming, and South Dakota), whereas the US2 region had sites from Montana, southern Idaho, and northern Utah. The US3 region encompassed sites from the Pacific Northwest (southeastern Washington and northeastern Oregon).

DNA isolation and molecular marker analysis

Eight seeds per accession were planted and leaf tissue was harvested from each seedling and bulked for DNA extraction. DNA was extracted from 35 mg of leaf tissue following the protocol described by Riera-Lizarazu et al. (2000). Twenty wheat chloroplast (WCt) microsatellite markers (Ishii et al. 2001) were used to characterize the chloroplast genome and 24 Gaterslaben wheat microsatellite (gwm) markers (Roder et al. 1998) were used to evaluate the nuclear genome. Out of the 24 nuclear markers analyzed, marker gwm 165 and gwm 205 consistently

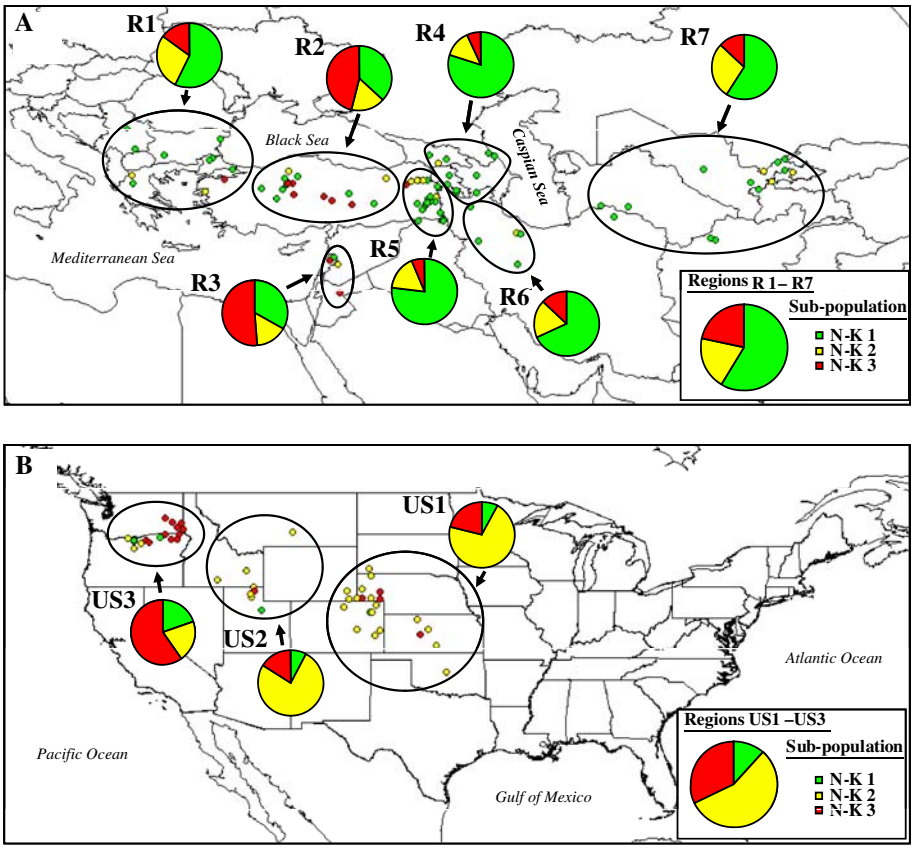

Fig. 2 Maps showing the distribution of *Ae. cylindrica* collections from various regions and their inferred membership to various subpopulations (N-K1, N-K2, and N-K3). **a** Map with locations of accessions collected in the native distribution of *Ae. cylindrica*. Regions are labeled as R1 to R7. **b** Map with approximate locations of accessions collected in the USA. Regions in the USA are labeled as US1, US2, and US3. Pie charts show the collective proportional assignment of individuals to subpopulations N-K1 (*green*), N-K2 (yellow), and N-K3 (*red*). Insets show pie charts with the average population membership for all individuals collected in *Ae. cylindrica*'s native range (**a**) and in the USA (**b**) (see Table 3)

detected two loci, increasing the total number of nuclear genome loci for analysis to 26.

For microsatellite marker assays, one primer was labeled with a fluorescent dye [6-carboxyfluorescein (FAM), or 4,7,2′,4′,5′,7′-hexachloro-6-carboxyfluroscein (HEX), or 4,7,2′,7′-tetrachloro-6-carboxyfluroscein (TET)]. Polymerase chain reactions (PCR) were carried out in 10-µl reactions containing 0.03 units *Taq* polymerase with 1× PCR buffer containing 1.5 mM $MgCl_2$ (Qiagen, Valencia, CA, USA), 2% sucrose in 0.04% cresol red, 0.2 mM of each dNTP, and 0.2 µM of each primer. The PCR consisted of an initial DNA-denaturing step at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 50–60°C (depending on primers) for 1 min, and extension at 72°C for 1 min, with a final step of extension at 72°C for 10 min. Fragment analysis was carried out using either ABI Prism® 377 DNA Sequencer or ABI Prism® 3100 Genetic Analyzer at the Core Labs, Center for Genome Research and Biocomputing, Oregon State University. ABI GeneScan® 2.1 and Genotyper® 2.0 software (Applied Biosystems, Foster City, CA, USA) were used to size fragments based on an internal lane standard [*n*,*n*,*n*′,*n*′-tetramethyl-6-carboxyrhodamine (TAMRA) or 6-carboxy-x-rhodamine (ROX)].

Statistical analyses

Descriptive statistics, such as the number and frequency of alleles were calculated using PowerMarker (Liu and Muse 2005) and CONVERT (Glaubitz 2004). PowerMarker also was used to generate a genetic distance (dissimilarity) matrix based on the proportion of shared alleles (Bowcock et al. 1994). The genetic distance matrices were then subjected to the neighbor-joining method (Saitou and Nei 1987) of tree formation. MEGA 4.0 (Kumar et al. 2004; Tamura et al. 2007) was used to produce graphical trees.

Indices of diversity that compensate for sampling size disparities were calculated on a per region basis. These indices were the unbiased expected heterozygosity or gene diversity (Nei 1978) and allelic richness calculated by rarefaction using HP-RARE 1.0 (Kalinowski 2004, 2005). For chloroplast microsatellite marker data, indices of diversity for *Ae. cylindrica* accessions with C- and D-plastome types were calculated separately. Analysis of molecular variance (AMOVA) and indices of population differentiation, F_{ST}, were calculated using Arlequin 3.1 (Excoffier et al. 2005). The statistical significance of pair-wise F_{ST} estimates was tested by performing 1,000 iterations of re-sampling.

Genetic structure analyses

Analyses of genetic structure were performed using Bayesian clustering methods implemented in Structure 2.1 (Falush et al. 2003; Pritchard et al. 2000) and Structurama (Huelsenbeck and Andolfatto 2007). These methods use multilocus genotypes to infer the fraction of an accession's genetic ancestry (Q) that belongs to a subpopulation or cluster, for a given number of subpopulations (K). Analyses with Structurama suggested that $K = 3$ was appropriate for our nuclear microsatellite data. With Structure 2.1, we used a model with $K = 3$ that assumed admixture and correlated allele frequencies between populations. We ran simulations involving 10 iterations of 80,000 steps after 80,000 steps of burn-in. An accession was assigned to a cluster if at least 75% of its genome was estimated to belong to that cluster. Individuals which did not meet this criterion were classified as being admixed. We also performed population assignments using a model of $K = 3$ with Structurama (Markov chain Monte Carlo of 10,000 cycles). The subpopulation assignments with Structure 2.1 and Structurama were concordant (assignments are shown in the supplementary Table 1).

For our chloroplast microsatellite data, Structurama suggested that $K = 3$ was appropriate. As with the nuclear marker dataset population assignments were performed with both Struture 2.1 (model with $K = 3$, no admixture, correlated allele frequencies and iterations of 80,000 steps after 80,000 steps of burn-in) and Structurama (model with $K = 3$ and Markov chain Monte Carlo of 10,000 cycles). The population assignments with Structure 2.1 and Structurama were concordant (assignments are shown in the supplementary Table 1).

Results

Genetic diversity and structure based on nuclear microsatellite markers

The genetic diversity of *Ae. cylindrica* in adventive locations in the USA and its native range was low compared to that of its diploid progenitors, *Ae. tauschii* and *Ae. markgrafii*. A list of nuclear microsatellite markers and a summary of allele frequencies can be found in supplementary Table 2. The average gene diversity (H_E) for *Ae. cylindrica* was 0.24, whereas the gene diversity estimates for *Ae. tauschii* and *Ae. markgrafii* were 0.68 and 0.38, respectively. When diversity was compared across geographic regions, accessions from the R4 and R5 regions exhibited the highest values of gene diversity ($H_E = 0.29$ and 0.28, respectively; Table 1). Accessions from regions R4 and R5 also showed the highest values of allelic richness. These results indicated that the greatest allelic diversity is present among accessions from Transcaucasia (region R4) and eastern Turkey (region R5). Using these measures, accessions from regions R3, R6, and R7 were

Table 1 Summary of diversity indices for nuclear genomes by geographic region

Diversity index[a]	*Ae. cylindrica* by geographic region[b]										*Ae. tauschii*	*Ae. markgrafii*
	R1	R2	R3	R4	R5	R6	R7	US1	US2	US3		
n	13	19	4	12	29	5	15	35	12	29	5	3
H_E	0.2570	0.2437	0.2271	0.2896	0.2804	0.1849	0.2221	0.2259	0.2291	0.2196	0.6828	0.3849
a_g	1.2471	1.2343	1.2184	1.2784	1.2696	1.1778	1.2135	1.2172	1.2203	1.2111	1.6555	1.3681

[a] *n* is the number of accessions. H_E is the unbiased expected heterozygosity or gene diversity (Nei 1978). a_g is the allelic richness of a sample calculated by rarefaction (Kalinowski 2004)

[b] Geographic regions correspond to those in Fig. 2

Table 2 AMOVA of *Aegilops cylindrica* diversity

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage of variation
Among regions	9	198.112	0.57256	16.32
Within regions	336	986.492	2.93599	83.68
Total	345	1,184.604	3.50855	
Fixation index		F_{ST}	0.16319*	

AMOVA Analysis of molecular variance according to Excoffier et al. (1992)

* F_{ST} values were significant at $p < 0.001$

found to have the least amount of diversity, whereas accessions from regions R1 and R2 had intermediate levels of diversity (Table 1). Accessions from the USA (regions US1, US2, and US3) showed levels of allelic diversity that were comparable to accessions from the R3 and R7 regions.

Global and pair-wise estimates of F_{ST} suggested that there was significant population differentiation among *Ae. cylindrica* from the various geographical regions that were sampled (supplementary Table 3). Analysis of molecular variance (AMOVA) showed that most of the genetic variation (84%) was partitioned within geographic regions rather than among regions (16%) (Table 2). Bayesian clustering implemented in Structure 2.1 and Structurama were used to better understand population structure. Analyses with Structurama suggested that *Ae. cylindrica* accessions used in this study could be divided into three subpopulations ($K = 3$). Members of these three subpopulations (labeled N-K1, N-K2, and N-K3) were present in adventive as well as native sites of *Ae. cylindrica*'s distribution (Fig. 2; supplementary Table 1). The majority of accessions from *Ae. cylindrica*'s native range belonged to subpopulation N-K1, whereas the majority of genotypes collected in the USA belonged to subpopulation N-K2. Genotypes from regions R1, R4, R5, R6, and R7 had comparable subpopulation membership, where most belonged to subpopulation N-K1 (Table 3; Fig. 2). Genotypes from R2 and R3 region had comparable population structure, where most accessions belonged to subpopulation N-K3. In the USA, genotypes from the US1 and US2 regions had comparable subpopulation memberships, where most belonged to subpopulation N-K2. On the other hand, most genotypes in the US3 region belonged to the N-K3 subpopulation (Fig. 2).

Table 3 Average cluster membership of 173 *Ae. cylindrica* accessions from 12 geographic regions

Region[a]	Cluster			No. of accessions
	N-K1	N-K2	N-K3	
R1	0.57	0.28	0.15	13
R2	0.37	0.17	0.46	19
R3	0.33	0.16	0.51	4
R4	0.80	0.13	0.07	12
R5	0.77	0.17	0.06	29
R6	0.68	0.19	0.13	5
R7	0.59	0.28	0.13	15
Subtotal	0.59	0.20	0.22	97
US1	0.08	0.71	0.21	35
US2	0.08	0.76	0.16	12
US3	0.20	0.20	0.60	29
Subtotal	0.12	0.56	0.32	76
Grand Total	0.35	0.38	0.27	173

[a] Geographic regions correspond to those in Fig. 2

In a distance-based cladogram, excluding admixed individuals, *Ae. cylindrica* accessions were grouped in a single cluster. Accessions of *Ae. markgrafii* were more closely related to *Ae. cylindrica* than to *Ae. tauschii* (Fig. 3). The *Ae. tauschii* accessions G435 (TU-4) and

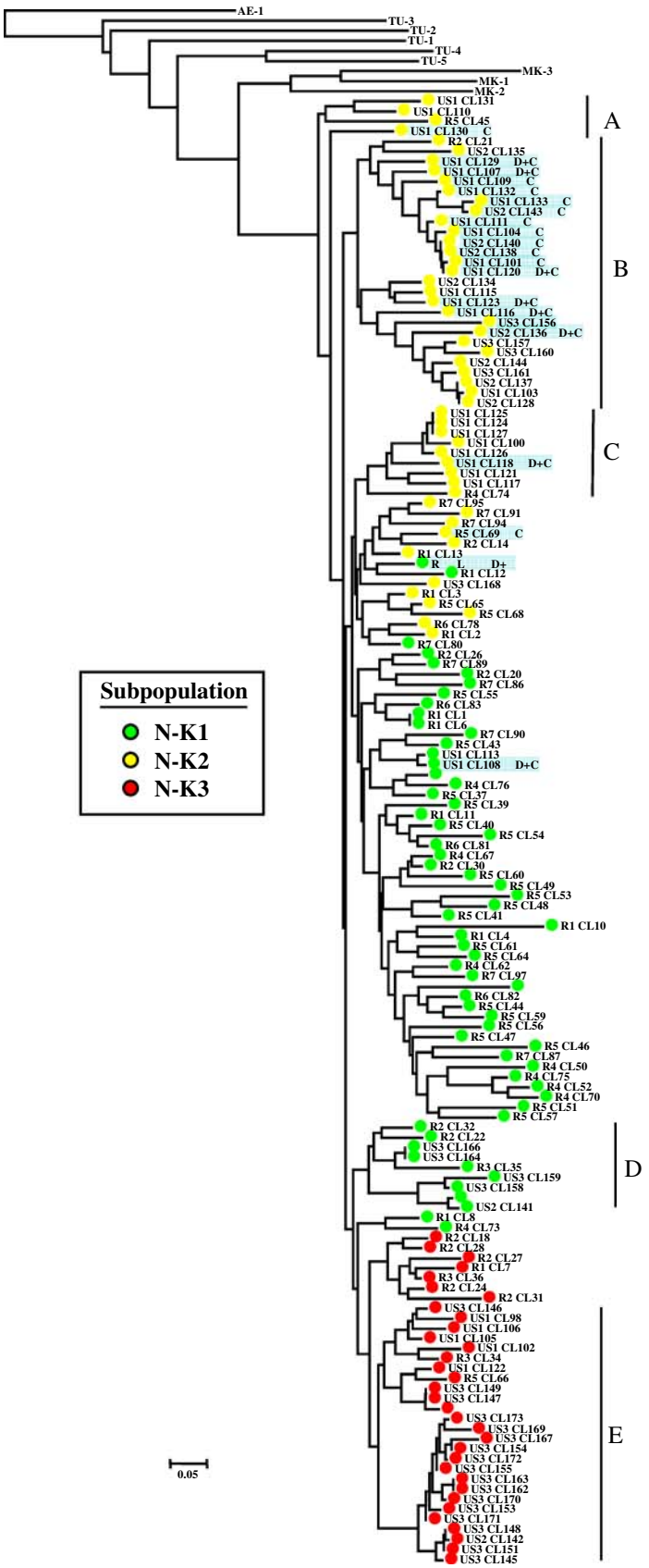

Fig. 3 Neighbor-joining tree showing nuclear genetic relatedness between *Ae. cylindrica* and its relatives. The prefixes used before the name of each accession stand for the following: R or US = *Ae. cylindrica*; AE = *T. aestivum*, MK = *Ae. markgrafii*, and TU = *Ae. tauschii.* Prefix for *Ae. cylindrica* accessions includes information about their region of origin. For example, R1_CL, would indicate *Ae. cylindrica* from the R1 region and US1_CL, would indicate *Ae. cylindrica* from the US1 region. Subpopulation membership of each accession based on Bayesian clustering is shown by a colored circle to the left of each sample label. A *green* circle marks an accession that belongs to subpopulation N-K1, a *yellow* circle marks an accession from the N-K2 subpopulation, and a *red* circle marks an individual from the N-K3 subpopulation. The labels of accessions followed by a 'C' or 'D + C' mark accession with C-type plastome and accessions with both D- and C-type plastomes, respectively. The labels of accessions with C-type or D- plus C-type plastomes are also highlighted in light *blue*. Clusters (A, B, C, D, and E) of closely related *Ae. cylindrica* accessions from the USA are marked with *vertical bars* (on the right)

G5792 (TU-5), most closely related to *Ae. cylindrica*, are taxonomically classified as *Ae. tauschii* ssp. *tauschii* (Fig. 3; supplementary Table 1). The *Ae. tauschii* ssp. *strangulata* accessions G1278 (TU-2) and G1273 (TU-3) were found to group closer to wheat (*T. aestivum*). These groupings in the distance-based tree showed some correspondence to the Bayesian-based clustering (Fig. 3).

Genetic diversity and structure based on chloroplast microsatellite markers

Of the 173 *Ae. cylindrica* accessions analyzed, 12 accessions were found to have more than one allele at some chloroplast microsatellite loci (supplementary Table 1). These heterogeneous samples were de-bulked and re-analyzed. Thus, the total number of *Ae. cylindrica* samples analyzed with chloroplast markers increased to 185. A list of chloroplast microsatellite markers and a summary of allele frequencies can be found in supplementary Table 4. A distance-based cladogram showed three distinct clusters. These clusters corresponded to three cytoplasmic types (or plasmon types) in our samples (Fig. 4). Plasmon type B was represented by a single common wheat (*T. aestivum*) accession (Chinese Spring—AE-1). Plasmon type D was represented by 19 *Ae. tauschii* and 161 *Ae. cylindrica* accessions. Plasmon type C was represented by 8 *Ae. markgrafii* and 24 *Ae. cylindrica* accessions. Thus, the frequency of *Ae. cylindrica* samples with D-type plastomes (87%) was greater than the frequency of *Ae. cylindrica* with C-type plastomes (13%). Interestingly, the frequency of *Ae. cylindrica* accessions with a C-type plastome in the USA (24.3%) was substantially greater than in its native area of distribution (3%). The majority of the *Ae. cylindrica* with C-type plastome were collected in the US1 (16) and US2 (4) regions. The R5 and US3 regions contributed a single accession each, whereas R7 had two *Ae. cylindrica* accessions with the C-type plastome.

Within the C-type cluster, the *Ae. markgrafii* var. *polyathera* accession G758 (MK-3) was closely related to C-type *Ae. cylindrica* (Fig. 4). The *Ae. cylindrica* accessions CO-18 (US1-CL111) did not group with other C-type *Ae. cylindrica* and was closely related to other *Ae. markgrafii* accessions. In the D-type cluster, *Ae. tauschii* ssp. *tauschii* accessions 84TK154-043 (TU-1), TA10143 (TU-15), TA10145 (TU-17), and TA1588 (TU-19) were more closely related to D-type *Ae. cylindrica* than to other *Ae. tauschii* accessions (Fig. 4).

As was the case with nuclear markers, chloroplast markers showed that plastome diversity in *Ae. cylindrica* was lower than that of its diploid progenitors. The average gene diversity for D-type *Ae. cylindrica* was 0.10, whereas the gene diversity for *Ae. tauschii* was 0.45. Gene diversity and allelic richness estimates showed that genotypes from northwestern Iran (region R6) were the most diverse (Table 4). Using these measures, accessions from regions R1, R2, and R5 were found to have the least diversity, whereas accessions from regions R3, R4 and R7 had intermediate levels of diversity. Accessions from the USA (regions US1, US2, and US3) showed gene diversity values ranging from 0.08 to 0.13 (Table 4). *Ae. cylindrica* with C-type plastome (C-type *Ae. cylindrica*) had an average gene diversity value of 0.09, whereas the gene diversity for *Ae. markgrafii* was 0.31 (Table 5). Genotypes from the USA showed comparable gene diversity ($H_E = 0.09$) to genotypes from *Ae. cylindrica*'s native range ($H_E = 0.07$) (Table 5).

When model-based clustering was used to study the relationship between the plastomes of *Ae. tauschii*, *Ae. markgrafii*, and *Ae. cylindrica*, the analysis suggested that individuals with D-type plastomes (*Ae. tauschii* and D-type *Ae. cylindrica*) could be divided into two subpopulations (D-K1 and D-K2). Fifteen *Ae. tauschii* accessions belonged to the D-K2 subpopulation and four belonged to the D-K1 subpopulation. All of the D-type *Ae. cylindrica* accessions belonged to the D-K1 cluster (Fig. 4) including the four *Ae. tauschii* ssp. *tauschii* accessions [84TK154-043 (TU-1), TA10143 (TU-15), TA10145 (TU-17), and TA1588 (TU-19)] that were closely related to D-type *Ae. cylindrica* in the chloroplast marker-based cladogram (Fig. 4). Model-based clustering suggested that individuals with C-type plastomes (*Ae. markgrafii* and C-type *Ae. cylindrica*) belonged to a single population (C-K1).

Discussion

The origin of *Ae. cylindrica*

Aegilops cylindrica has formed through amphidiploidization of a hybrid or hybrids between *Ae. tauschii* and *Ae. markgrafii* (syn. *Ae. caudata*) (Chennaveeraiah 1960; Jaaska 1981; Johnson 1967; Kihara and Matsumura 1941). There are four morphological varieties of *Ae. tauschii* that are grouped in two subspecies—*Ae. tauschii* spp. *strangulata* (var. *strangulata*) and *Ae. tauschii* spp. *tauschii* (var. *typica*, var. *meyeri*, and var. *anathera*) (Eig 1929; Kihara and Tanaka 1958; Tanaka 1983). Cytogenetic and molecular-based analyses suggested that the D genomes of *Ae. cylindrica* and *T. aestivum* were contributed by different biotypes of *Ae. tauschii* (Badaeva et al. 2002; Caldwell et al. 2004). The D genome of hexaploid wheat has been shown to be more closely related to the D genome of *Ae. tauschii* ssp. *strangulata* than to *Ae. tauschii* ssp. *tauschii* (Dvorak et al. 1998; Lubbers et al. 1991; Pestsova et al. 2001), whereas the D-type plastome and the D genome of *Ae. cylindrica* are more closely related to

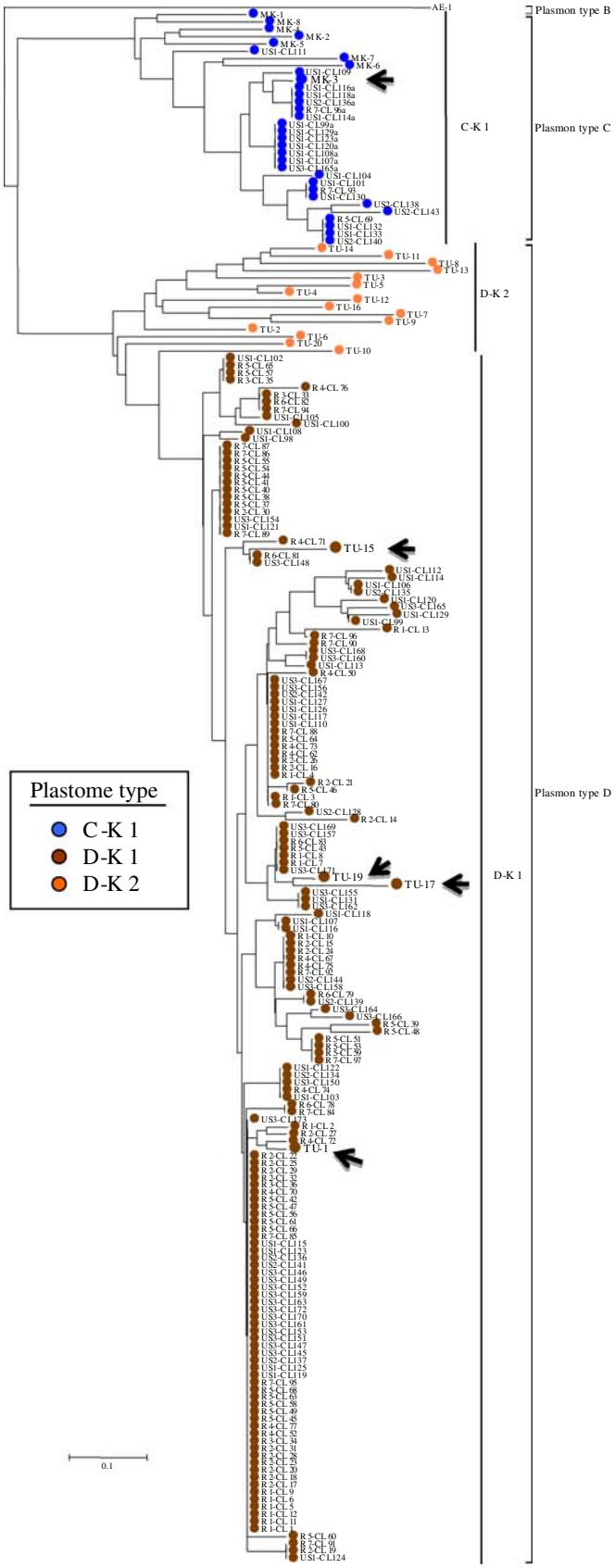

Fig. 4 Neighbor-joining tree showing chloroplast marker-based genetic relatedness between *Ae. cylindrica* and its relatives. The prefixes used before the name of each accession stand for the following: R or US = *Ae. cylindrica*; AE = *T. aestivum*, MK = *Ae. markgrafii*, and TU = *Ae. tauschii.* Prefix for *Ae. cylindrica* accessions includes information about their region of origin. For example, R1-CL would indicate *Ae. cylindrica* from the R1 region and US1-CL would indicate *Ae. cylindrica* from the US1 region. Clusters corresponding to plasmon types B, C, and D are shown with *vertical bars* on the right. Subpopulation membership of each accession based on Bayesian clustering is shown by a colored circle to the left of each sample label. *Ae. markgrafii* and all C-type *Ae. cylindrica* belonged to one subpopulation (C-K1; *blue* circles). The position of the *Ae. markgrafii* accession G 758 (MK-3) that was most closely related to *Ae. cylindrica* is marked with an *arrow*. *Ae. tauschii* was subdivided into two subpopulations [D-K1 (*brown circles*) and D-K2 (*orange circles*)]. All of the D-type *Ae. cylindrica* accessions belonged to subpopulation D-K1. The position of *Ae. tauschii* accessions 84TK154-043 (TU-1), TA10143 (TU-15), TA10145 (TU-17), and TA1588 (TU-19) that were most closely related to *Ae. cylindrica* are marked with *arrows*

Table 4 Summary of diversity indices for D-type plastomes by geographic region

Diversity index[a]	*Ae. cylindrica* by geographic region[b]										*Ae. tauschii*
	R1	R2	R3	R4	R5	R6	R7	US1	US2	US3	
n	13	19	4	12	28	5	14	28	9	29	19
H_E	0.0692	0.0596	0.0917	0.1038	0.0730	0.1500	0.0983	0.1325	0.0917	0.0793	0.4494
a_g	1.1662	1.1623	1.1482	1.2519	1.1648	1.3098	1.2244	1.2984	1.2050	1.2016	2.1619

[a] *n* is the number of accessions. H_E is the unbiased expected heterozygosity or gene diversity (Nei 1978). a_g is the allelic richness of a sample calculated by rarefaction (Kalinowski 2004)

[b] Geographic regions correspond to those in Fig. 2

Table 5 Summary of diversity indices for C-type plastomes by geographic region

Diversity index[a]	*Ae. cylindrica* by geographic region[b]		*Ae. markgrafii*
	Native	USA	
n	3	21	8
H_E	0.0667	0.0928	0.3125
a_g	1.1000	1.1809	1.7097

[a] *n* is the number of accessions. H_E is the unbiased expected heterozygosity or gene diversity (Nei 1978). Allelic richness was calculated by rarefaction (Kalinowski 2004)

[b] Geographic regions correspond to those in Fig. 2

Ae. tauschii ssp. *tauschii* than to *Ae. tauschii* ssp. *strangulata* (Gandhi et al. 2005). In the present study, model-based clustering of chloroplast marker data suggested the presence of two plastome subpopulations, D-K1 and D-K2, in *Ae. tauschii* (Fig. 4). All *Ae. tauschii* ssp. *strangulata* and most *Ae. tauschii* spp. *tauschii* accessions belonged to the D-K2 subpopulation but four *Ae. tauschii* ssp. *tauschii* accessions and all D-type *Ae. cylindrica* accessions belonged to subpopulation D-K1. In the distance-based cladograms using chloroplast and nuclear microsatellite markers (Figs. 3 and 4), *Ae. tauschii* spp. *tauschii* accessions also were found to be more closely related to *Ae. cylindrica* than *Ae. tauschii* ssp. *strangulata.* Thus, our present study confirms the observations made in an earlier study (Gandhi et al. 2005) and indicates that *Ae. tauschii* spp. *tauschii* contributed one of its plastome types and its D genome to *Ae. cylindrica*.

In a previous study with chloroplast and nuclear microsatellite markers (Gandhi et al. 2005), the reported genetic differentiation of *Ae. markgrafii* from the western and eastern regions of its distribution (Ohta 2000, 2001) was not evident. In the present study, model-based clustering of chloroplast marker data also failed to show this pattern of differentiation in *Ae. markgrafii* (Fig. 4). All accessions with C-type plastomes that were studied belonged to a single subpopulation (C-K1). The *Ae. markgrafii* var. *polyathera* accession G 758 (MK-3, unknown origin) that we had not studied previously, had the most closely related plastome to C-type *Ae. cylindrica*. This accession has been used in other studies (Mason-Gamer et al. 1998) but its collection site is unknown. Besides accession G 758, the *Ae. markgrafii* var. *polyathera* accession KU5852 (MK-6, north central Turkey) and *Ae. markgrafii* var. *markgrafii* accession KU5864 (MK-7, western Turkey) also appeared to be closely related to C-type *Ae. cylindrica*.

Although we could not definitively determine the source of the C-type plastome or the C genome of *Ae. cylindrica*, genotypes from the eastern region of *Ae. markgrafii*'s distribution (Fertile Crescent arc) are the likely candidates. *Ae. markgrafii* can be found in the northeastern Mediterranean basin, western and central Turkey, and along most of the Fertile Crescent (Ohta 2001; Slageren 1994). *Ae. tauschii* ssp. *strangulata* is found in Transcaucasia and in coastal areas of eastern Caspian Iran, whereas *Ae. tauschii* ssp. *tauschii* is distributed from eastern Turkey to China and Pakistan. Thus, northern Syria, southeastern Turkey, and northern Iraq encompass an area, where the distributions of *Ae. markgrafii* and *Ae. tauschii* ssp. *tauschii* can overlap. If the distributions of *Ae. markgrafii* and *Ae. tauschii* were not significantly different in the past, then this area of overlap is likely to be where *Ae. cylindrica* arose. Our results are consistent with this argument because we found three accessions of *Ae. tauschii* ssp. *tauschii* [TA10143 (TU-15), TA10145 (TU-17), and TA1588 (TU-19)], collected in this region of overlap, with plastomes that were the most closely related to D-type *Ae. cylindrica*. Although our analysis suggests that *Ae. cylindrica* probably arose along the Fertile Crescent, our analysis also shows that the center of genetic diversity of this species now encompasses a larger area including northern Iraq, eastern Turkey, and Transcaucasia.

Recently, Caldwell et al. (2004) suggested that *Ae. cylindrica* has formed recurrently through multiple hybridization events. In this study, D- and C-type *Ae. cylindrica* accessions associated in clusters of closely

related individuals in the nuclear marker tree, most of which, belonged to subpopulation N-K2. Thus, the formation of this subpopulation may be due to reciprocal hybridization between its diploid progenitors or cytoplasmic introgression from *Ae. markgrafii* after the formation of *Ae. cylindrica*. D-type *Ae. cylindrica* accessions of the N-K1 and N-K3 population also formed clusters of closely related individuals that may correspond to independent hybridization events. Thus, our study supports the idea that *Ae. cylindrica* may have formed recurrently.

Though *Ae. cylindrica* origin is unknown, its probable origin along the Fertile Crescent and its notorious weediness in cereal fields, compared to other species of the genus *Aegilops*, suggest that the successful establishment of *Ae. cylindrica* may have coincided with the domestication of crops and the rise of agriculture in this region of the world (Lev-Yadun et al. 2000; Salamini et al. 2002). In this context, more detailed phylogenetic and population genetics studies will be needed to clarify the evolutionary history of *Ae. cylindrica* with respect to other species of the *Triticum/Aegilops* complex (Huang et al. 2002).

Genetic structure

In phylogenetic trees based on genetic distances, no clear relationship between cladogram clustering and geographic origin was observed. Instead accessions from different geographic regions were interspersed in trees suggesting that the *Ae. cylindrica* accessions that we studied were not structured along the geographic regions outlined in this study. This observation is consistent with the AMOVA analysis which showed that most of the genetic variation was present within regions rather than among regions. Thus, the structure of *Ae. cylindrica* populations reflect the wide dispersal of genetically distinct accessions (based on Bayesian clustering) between the geographic regions that we delineated in this study. A marked difference in the genetic makeup was observed between *Ae. cylindrica* in its native range and the USA. In its native range, genotypes belonging to the N-K1 subpopulation were the most numerous followed by accessions belonging to the N-K2 and N-K3 subpopulations (Table 3). Accessions belonging to the N-K1 and N-K2 subpopulations were present in all regions (their geographic distribution overlapped), whereas genotypes belonging to the N-K3 subpopulation were absent from regions R4, R6, and R7. Thus, genotypes belonging to the N-K1 and N-K2 subpopulations appear to be widely distributed, whereas genotypes of the N-K3 subpopulation appear to be concentrated in central Turkey and its vicinity. In the USA, genotypes belonging to the N-K2 subpopulation were the most numerous followed by genotypes belonging to the N-K3 subpopulation. Individuals belonging to the N-K1 subpopulation were rare. Although the geographic distribution of all genotypes overlapped, the composition of genotypes in the US3 region was distinct from those in regions US1 and US2. The frequency of genotypes belonging to the N-K3 subpopulation was greater in the US3 regions than in regions US1 and US2. Conversely, the frequency of genotypes belonging to the N-K2 subpopulation was high in regions US1 and US2 relative to region US3. Thus, accessions of the N-K2 subpopulation, in general, appear to be better adapted to the Great Plains and western regions of the USA, whereas genotypes of the N-K3 subpopulation appear to be better adapted to the Pacific Northwest. On the other hand, as discussed below, the pattern of distribution of various genotypes may reflect the diffusion of founder populations.

Ae. cylindrica in the USA

It has been argued that *Ae. cylindrica* may have been initially introduced into the state of Kansas, in the 1800s, as a contaminant in wheat stocks brought by Russian Mennonite immigrants (Johnston and Parker 1929). Other introductions of *Ae. cylindrica* have also been attributed to sporadic escapes, in the early 1900s, from experimental plots (Kennedy 1928). However, most agree that the rapid spread of jointed goatgrass in Kansas and elsewhere, in the early 1900s, was likely due to the aggressive introduction and promotion of winter wheat from southern Russia and Ukraine by the U.S. Department of Agriculture, seed dealers, and/or private individuals (Donald and Ogg 1991; Johnston and Heyne 1960; Mayfield 1927; Morrison et al. 2002). Although *Ae. cylindrica* may have been introduced at multiple times and locations, our study of its population structure suggests the spread of only a sub-sample of the genetic variation in this species. In the genetic distance-based cladogram, accessions from the USA formed clusters of very closely related genotypes (Fig. 3; clusters A, B, C, D, and E) that were interspersed with few accessions from *Ae. cylindrica*'s native range. This pattern of association suggests that *Ae. cylindrica* populations in the USA spread through a few founder genotypes. Spread through a founder effect was clearly evident for C-type *Ae. cylindrica* from the USA (Fig. 3; clusters A, B, and C) where all except one accession (CO-13) belonged to subpopulation N-K2.

Low non-significant F_{ST} values between accessions from the US1 and US2 regions suggest a lack of population differentiation between accessions from these regions. On the other hand, statistically significant pair-wise F_{ST} estimates were observed between the US1 and US3 regions and between the US2 and US3 regions (supplementary Table 3). This was consistent with Bayesian clustering methods that showed that most accessions from the US3 region belonged to the N-K3 population, whereas most

accessions from the US1 and US2 regions belonged to the N-K2 subpopulation. This pattern of population structure might reflect two independent introductions of *Ae. cylindrica* in the USA—individuals of the N-K2 subpopulation being introduced into the US1 and US2 regions and individuals of the N-K3 subpopulation being introduced into the US3 region. On the other hand, a relatively heterogeneous collection of *Ae. cylindrica* genotypes may have been simultaneously introduced into regions US1, US2, and US3 and the pattern of population structure today reflects the differential adaptation of *Ae. cylindrica* to these different agro-ecological zones.

It has been argued that knowing the origin of jointed goatgrass might guide the discovery of candidate organisms for its biological control (Pester et al. 2003). Our analysis has shown that accessions from the USA are related to genotypes that are widely distributed in jointed goatgrass's native range making a definitive link to a specific locale difficult if not impossible. If wheat imports from southern Russia led to the introduction of jointed goatgrass into the USA, a comprehensive survey of accessions from eastern Europe might help address this issue further.

C- and D-type plastomes in *Aegilops cylindrica*

Analyses with chloroplast microsatellite markers confirmed our earlier study (Gandhi et al. 2005), where both C- and D-type plastomes were found in *Ae. cylindrica*. Using a larger collection of *Ae. cylindrica* in this study, the relative frequency of the C- and D-type plastomes could be assessed. The C-type plastome was less frequent than the D-type plastome in *Ae. cylindrica*'s native distribution and in the USA. This difference was dramatic in the native range, where only 3% of accessions carried the C plastome type. In the USA, up to 24% of accessions had the C-type cytoplasm. These results suggest that the D-type plastome is, in general, favored over the C-type plastome but conditions in the USA (particularly regions US1 and US2) appear to be more favorable for C-type *Ae. cylindrica*. Studies with alloplasmic lines have shown that the cytoplasm can have significant effects on traits related to reproduction (Tsunewaki 1996; Tsunewaki et al. 2002). Since the great majority of C-type *Ae. cylindrica* from the USA belonged to a single subpopulation (N-K2), the high frequency of C-type *Ae. cylindrica* in the USA may be due to this favorable nucleo-cytoplasmic combination.

Genetic diversity and adaptation

Diversity indices for nuclear and chloroplast microsatellites showed lower levels of diversity in *Ae. cylindrica* compared to its diploid progenitors. Low levels of genetic diversity in *Ae. cylindrica* are consistent with other studies (Gandhi et al. 2005; Goryunova et al. 2004; Okuno et al. 1998; Pester et al. 2003) and have been interpreted to reflect the origin of *Ae. cylindrica*; namely, this allotetraploid formed from few relatively recent hybridization events between *Ae. tauschii* and *Ae. markgrafii* (Caldwell et al. 2004; Gandhi et al. 2005). Thus, *Ae. cylindrica* contains only a subset of the genetic variation present in its diploid progenitors. In addition, analyses of genetic structure suggest that only a sub-sample of the genetic variation in this species was introduced into the USA. Despite its relatively narrow genetic base, *Ae. cylindrica* shows broader geographic distribution than its progenitors and despite having suffered genetic bottlenecks in its introduction in the USA, it has become a very successful adventitious weed. It is interesting to note that a similar phenomenon is observed in *Ae. triuncialis* L. ($2n = 4x = 28$; genome UUCC), an allotetraploid with dimaternal origin from amphidiploidization of hybrids between *Ae. umbellulata* Zhuk. ($2n = 2x = 14$; genome UU) and *Ae. markgrafii* (Murai and Tsunewaki 1986; Vanichanon et al. 2003; Wang et al. 1997). Studies on molecular variation of *Ae. triuncialis* have also shown that this species has suffered a severe genetic bottleneck in its introduction into the USA (Meimberg et al. 2006). Nonetheless, *Ae. triuncialis* is a successful invader and a very serious noxious weed of rangelands in California (DiTomaso et al. 2001; Peters et al. 1996).

Although low levels of genetic diversity (additive genetic variance) are expected to result in the reduced capacity of species, such as *Ae. cylindrica* or *Ae. triuncialis* to adapt to new environments, non-additive genetic variation in the form of dominance, epistasis, and fixed heterosis may provide the potential for increased fitness and selective adaptation (Lee 2002). Furthermore, studies at the molecular level have shown that the combination of two divergent genomes in allotetraploids by interspecific hybridization induces genomewide changes in DNA methylation, sequence rearrangements and losses, gene silencing, chromatin modifications, and non-additive gene regulation (Adams and Wendel 2005). These changes are now believed to represent the molecular basis for de novo variation that have afforded newly formed allopolyploids evolutionary opportunities for adaptation and success (Chen 2007; Leitch and Leitch 2008; Wang et al. 2006). In this context, the successful adaptation of allotetraploids like *Ae. cylindrica* to various environments deserves additional study.

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